Could microorganisms be preserved in Mars gypsum? Insights from terrestrial examples

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ABSTRACT

Could the abundant sulfate salts on Mars contain microfossils and/or viable microorganisms? Here we report a variety of microorganisms trapped both as solid inclusions and as potentially viable halophilic and acidophilic prokaryotes and eukaryotes within fluid inclusions in Mars-analog gypsum. We have documented pennate diatoms, green algae, and prokaryotes in gypsum precipitated from acid (pH 1.8–4.6) saline (5%–28% total dissolved solids) waters at Salars Gorbea and Ignorado in an active volcanic terrain in the high Andes (4000+ m) of northern Chile. These salars are strikingly similar in geology and geochemistry to Mars. We propose that this discovery should serve as a model for fossilization of possible life on Mars and may inform methodologies used in future missions to Mars. Furthermore, the potential long-term viability of microorganisms within fluid inclusions in gypsum suggests the possibility of a living, yet isolated and likely dormant, microbiological community on Mars today.

INTRODUCTION

Sulfate minerals, including an abundance of hydrated calcium sulfates such as gypsum, have been documented on the martian surface since the 1970s with data from satellites, landers, and rovers (i.e., Bell, 1996; Bishop et al., 2004; Clark and Van Hart, 1981; Clark et al., 2005; Gendrin et al., 2005). High-resolution cameras on rovers have detected various morphological forms of hydrated calcium sulfates (i.e., Grotzinger et al., 2005; Metz et al., 2009; Squyres et al., 2004). Interpretations include (1) gypsum sand and silt grains reworked by winds and shallow surface waters (e.g., Benison and Bowen, 2006; Grotzinger et al., 2005; Metz et al., 2009; Squyres et al., 2004), (2) molds after displacive gypsum (Benison and Bowen, 2006; Herkenhoff et al., 2004), and (3) veins of gypsum (Squyres et al., 2012). The hydrated calcium sulfates on Mars are widely considered to have originally grown by acid saline shallow surface waters and groundwaters (Benison and Bowen, 2006; Benison and LaClair, 2003; Squyres et al., 2004, 2012). Some martian groundwaters precipitated sulfates early, such as in the case of the displacive gypsum molds (Benison and Bowen, 2006); other groundwaters formed vein sulfates as a later diagenetic process (Squyres et al., 2012). Many of the sulfates were likely physically reworked by wind and/or water, resulting in some of the dunes and ripples in modern martian sand and silt, and ripple-marked and cross-bedded ancient martian siltstones and sandstones (Grotzinger et al., 2005; Metz et al., 2009).

Despite the abundance of gypsum on Mars and the search for signs of life on Mars, few studies have been conducted about the fossilization potential of gypsum on either Earth or Mars. The few published studies focus on the identification of modern microorganisms living on or near terrestrial gypsum (Vitek et al., 2013) or use destructive high-magnification methods that cannot constrain the origin or entrapment mode of the biological material within ancient crystals (Panieri et al., 2008; Schopf et al., 2012). In contrast, studies of terrestrial halite (NaCl), another common chemical sediment, have documented prokaryotic and eukaryotic microorganisms and organic compounds within primary fluid inclusions (Conner and Benison, 2013; Lowenstein, 2012; Norton and Grant, 1988; Schubert et al., 2010, 2009b). Combined optical, chemical, and biological methods have been refined for the study of biological materials within fluid inclusions in halite. Careful in-situ petrographic study, combined with geochronological constraints, has shown that microorganisms in primary fluid inclusions in various recent and ancient halite were trapped during crystal growth from a surface water. Some of these entrapped microorganisms remain viable for at least tens of thousands of years (Schubert et al., 2009b; Mormile et al., 2003) and possibly for hundreds of millions of years (Vreeland et al., 2000, 2007). For this reason, evaporites may be considered to have exceptionally good fossilization potential. Although microorganisms have been documented in terrestrial halite and gypsum, the possibility of microorganisms in martian evaporites has been overlooked. For this reason, we must rely on Mars-analog evaporites to predict if martian evaporites might contain microorganisms.

Salars Gorbea and Ignorado are shallow acid saline lakes at 4000 m and 4200 m, respectively, located in the high Andes of the Atacama region of northern Chile (Fig. 1A). These acid salars are located ~200 km south of, and more than 1700 m higher in elevation than, the famous and larger Salar de Atacama. A survey of water geochemistry determined that of 84 Andean salars studied, only Salars Gorbea and Ignorado have low pH waters (Risacher et al., 2002). Surface waters and groundwaters at Salars Gorbea and Ignorado are SO_4-Na rich, with high amounts of

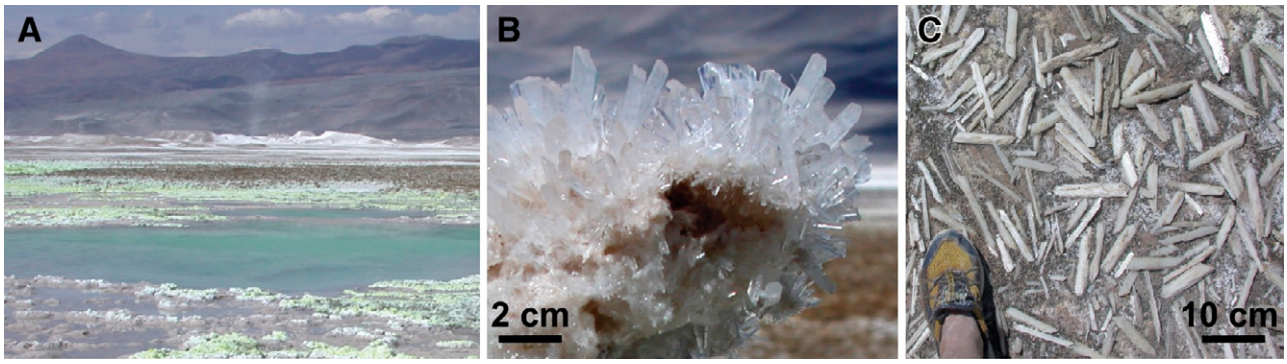

Figure 1. Gypsum at Salars Gorbea and Ignorado (high Andes of northern Chile). A: View of Salar Gorbea showing acid saline pool with white gypsum and yellow sulfur in foreground, white gypsum dunes in middle ground, and volcanoes in background. B: Bottom-growth gypsum crystals, taken from acid saline surface water. C: Bedding plane with large eolian-deposited gypsum grains. Note foot for scale.

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Mg, Cl, K, Al, and Ca (Risacher et al., 2002). Preliminary microbiological analyses of surface waters at Salar Gorbea, focused on prokaryotes, identified several classes of bacteria, showing that microbial life exists in these acid saline waters (Escudero et al., 2013). The acid saline waters precipitate a suite of minerals besides gypsum, including halite, native sulfur, jarosite, alunite, hematite, and clay minerals (Benison and Gonzalez, 2007). The salars are hosted on the flanks of active composite volcanoes of the Cerro Bayo complex. The host sediment is volcanic sand and gravel of andesitic and dacitic composition.

Salars Gorbea and Ignorado are extreme terrestrial environments in several ways. The setting is high in elevation and extremely arid, with very high winds, large diurnal and seasonal temperature ranges, and little macroscopic life. Water chemistry is also extreme; the pH is as low as 1.8 and salinity is as high as 28% total dissolved solids (Benison and Gonzalez, 2007). We propose that Salars Gorbea and Ignorado are among the best-known terrestrial analogs for Mars because they are similar in geochemistry, mineral composition, and sedimentary processes and products.

MATERIALS AND METHODS

Field work was conducted in March 2007 and consisted of documenting geological and geochemical characteristics and collecting samples. Sedimentary facies were mapped, with careful attention paid to observations of physical, chemical, and biological processes and the resulting products. Field geochemistry included measurements of surface water and groundwater depths, temperatures, pH, and total dissolved salinity. Mineral, sediment, rock, and water samples were collected.

Gypsum crystals were prepared minimally, by cleaving with a clean razor blade to obtain a thickness of 1–2 mm. Splitting crystals along cleavage planes resulted in smooth surfaces and made polishing unnecessary. Observations were made first with an Olympus SZX10 transmitted light stereo microscope, at magnification range of 6.3–63×, allowing for fluid inclusion assemblages to be determined. Higher magnification observations were made with an Olympus BX52 research petrographic microscope with transmitted, reflected, polarized, and ultraviolet-visible (UV-vis; excitation by combined 330 nm UV and 385 nm visible) light sources. The color of fluorescence relates to the type of organic matter (Mormile and Storrie-Lombardi, 2005; see the GSA Data Repository[1]). With the wavelengths of combined UV and visible light used for this study, prokaryotes fluoresced pale green, algae fluoresced blue, and carotenoids fluoresced red, orange, and pink (Conner and Benison, 2013). Magnification to 2000× into the gypsum crystal interior was conducted via long working distance objectives. Both microscopes are equipped with digital cameras and Spot Imaging Solution 5.0 software.

RESULTS

Gypsum Sedimentology

We have identified four types of gypsum at Salars Gorbea and Ignorado. Centimeter-scale, bottom-growth bladed gypsum crystals were observed growing in subaqueous clusters in shallow (to 2 m deep) surface-water pools (Fig. 1B). Abraded, randomly oriented bladed gypsum crystals found in dunes and on subaerially exposed surfaces, as well as unconsolidated in some surface pools, are interpreted as having originated as bottom-growth crystals and then reworked by strong winds as sand and gravel grains (Fig. 1C). Tiny (millimeter scale) needle-like gypsum crystals were found in subaerial crusts with native sulfur and tiny crystals of halite and other sulfate minerals. These efflorescent crusts formed as acid saline groundwater wicked to the surface by evaporation and underwent rapid precipitation (Smoot and Castens-Seidell, 1994). The fourth type of gypsum noted at Salars Gorbea and Ignorado was displacive lath-shaped crystals in the shallow subsurface sediments. For this study, we focus on the first two types of gypsum: the bottom-growth crystals (Fig. 1B) and the reworked grains (Fig. 1C). Both originated by precipitation from shallow surface waters and both contain abundant and large primary fluid inclusions that are remnants of these shallow surface waters.

Fluid Inclusion Petrography

Primary fluid inclusions are abundant and relatively large in gypsum from Salars Gorbea and Ignorado. They are as long as 100 µm and contain all-liquid, liquid-gas, liquid-solid, or liquid-gas-solid. Primary fluid inclusion assemblages, aligned in planes parallel to crystal faces, are interpreted to have formed from parent surface waters. Bottom-growth gypsum crystals host primary fluid inclusions, which represent modern acid saline pool fluids. Similar primary fluid inclusions in eolian transported gypsum grains originated as bottom-growth crystals before they were reworked by wind (Benison and Gonzalez, 2007), so they also represent surface acid saline fluids.

Microorganisms in Gypsum

Pennate diatoms exist as solid inclusions and within primary fluid inclusions (Figs. 2A and 2B). Due to their relatively large size, ~30 µm, they are readily recognized. They appear to represent one species, based on consistent morphology and size. Some clusters of dozens of diatoms appear pristine, suggesting that they had been living in the salar pool immediately before being trapped as the gypsum crystal grew (Fig. 2A). Other clusters contain abundant broken diatoms, indicating that they were abraded during wind transport and were physically deposited into the salar pool before being trapped in the growing gypsum (Fig. 2B). Diatoms entrapped within fluid inclusions are relatively rare. We have observed as many as three diatoms within an individual fluid inclusion. Like the solid inclusions of diatoms, some of the diatoms in fluid inclusions are pristine and some are abraded. Discrete parts of pristine diatoms fluoresce blue when exposed to UV-vis light (Fig. 2A2).

Suspect green algae are relatively abundant in Salar Gorbea and Ignorado gypsum. They appear as pale yellow and pale orange dimpled spheres and ovoids ranging in size from ~3 µm to 10 µm and are observed both as solid inclusions and in fluid inclusions (Figs. 2B, 2C, 2E, and 2H). They fluoresce blue (Fig. 2E). Some have a collapsed appearance, suggesting response to environmental stress (osmotic) and subsequent dormancy in a cyst state. Multiple spheres and ovoids are typically found together, with as many as ~20 in some individual inclusions. Many are quite similar in appearance to *Dunaliella* algae (Fig. 2D), documented in fluid inclusions in halite from ephemeral saline lakes elsewhere in the world, including in neutral Death Valley and Saline Valley in California (Lowenstein, 2012; Schubert et al., 2010) and acid Lake Magic in Western Australia (Conner and Benison, 2013). Suspect algae are typically accompanied by a clear, gel-like envelope (Fig. 2E), a red mass (Fig. 2C), or orange crystals. The clear envelope, red masses, and orange crystals fluoresce pale pink-orange, suggesting an organic compound such as glycerol and/or beta-carotene, both of which are commonly associated with halophilic algae (Schubert et al., 2010; Conner and Benison, 2013).

Prokaryotes are observed both as solid inclusions and within some fluid inclusions in gypsum (Fig. 2G). They appear as clear to pale yellow, high-relief, 1–2-µm-diameter spheres (Fig. 2G), and clear to pale yellow to clear, high-relief, 4–5-µm-long rods (Fig. 2H). All fluoresce pale green when exposed to UV-vis light. Some of these coccoid and bacilli-shaped cells appeared to move within the fluid inclusions, likely due to Brownian motion. These characteristics are consistent with prokaryotes (Conner and Benison, 2013; Lowenstein, 2012; Mormile and Storrie-Lombardi, 2005; Schubert et al., 2009a, 2009b, 2010). We interpret them as bacteria and/or archaea.

[1]GSA Data Repository item 2014220, supplemental information, is available online at www.geosociety.org/pubs/ft2014.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.

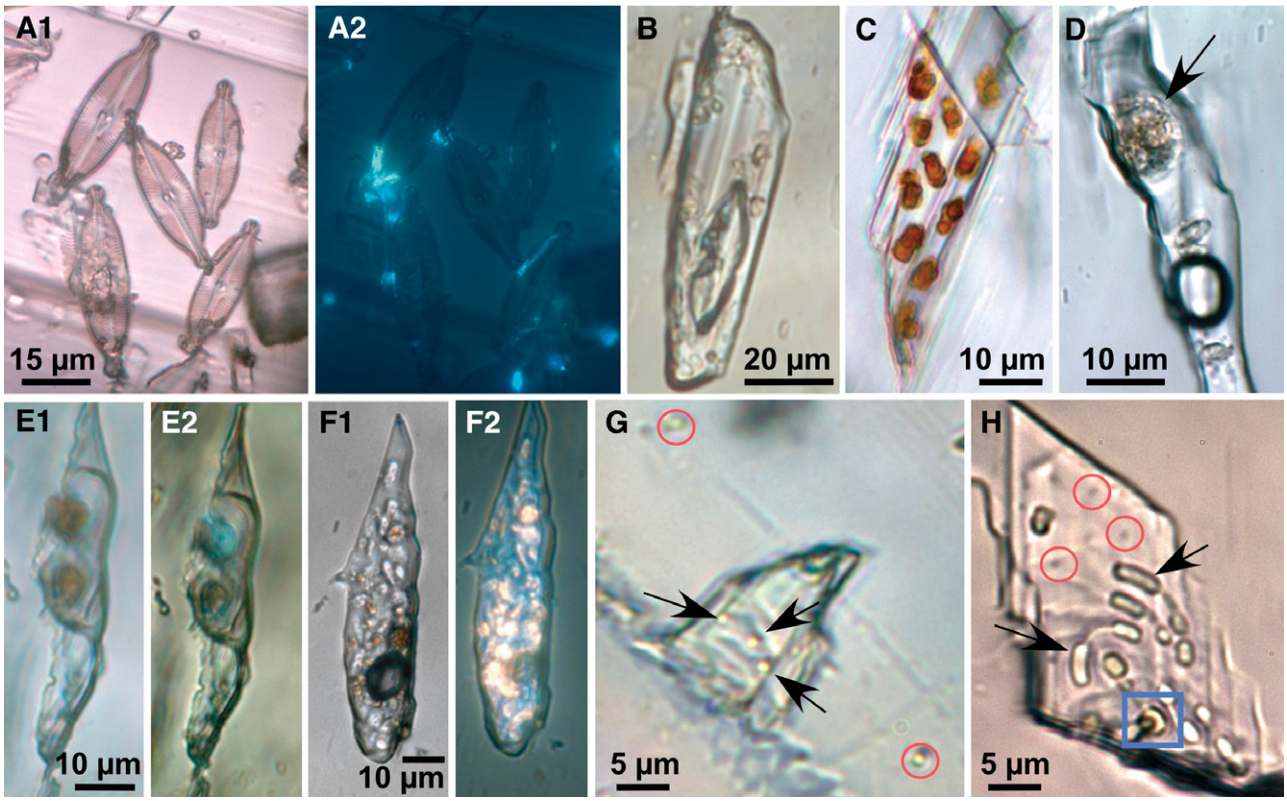

Figure 2. Microorganisms within gypsum from Salars Gorbea and Ignorado (northern Chile). A: Pennate diatoms in solid gypsum: A1 is in transmitted light and A2 is in ultraviolet-visible (UV-vis) light. B: Abraded pennate diatom in fluid inclusion, along with algal cells and prokaryotes. C: Algae (pale orange) and suspect beta-carotene (red) in fluid inclusion. D: Suspect *Dunaliella* algae (arrow) in fluid inclusion. E: Two algal cells surrounded by clear gel in fluid inclusion: E1 is in transmitted light and E2 is in combined transmitted light and UV-vis light. F: Prokaryotes and algae in fluid inclusion: F1 is in transmitted light and F2 is in UV-vis light. G: Cocci prokaryotes in fluid inclusion (arrows mark three cocci) and entrapped as solid inclusions (red circles). H: Fluid inclusion containing moving cocci prokaryotes (red circles), bacilli prokaryotes (arrows), and yellow algae (blue box).

DISCUSSION

Fluid inclusions may serve as host microenvironments for microorganisms. Previous workers have extracted inclusion fluids from halite and successfully cultured microorganisms, showing the viability of some microorganisms trapped within fluid inclusions (e.g., Lowenstein, 2012; Mormile et al., 2003). The optical methods we employ should be considered only a first step to guide further studies that may lead to chemical and DNA identification. In addition, the in-situ and nondestructive nature of the optical observations eliminates concerns about possible contamination.

Could microfossils and/or viable microorganisms be trapped in gypsum on Mars as they are in gypsum on Earth? It is likely that abundant sulfate sand grains on Mars contain fluid inclusions similar to those in the acid-precipitated bottom-growth and reworked gypsum we discuss here. We suggest that gypsum on Mars would have entrapped, as solid inclusions and within fluid inclusions, any microorganisms and/or organic compounds that were present in its parent waters. Therefore, fluid inclusions and solid inclusions hosted by salt minerals may be the best place to continue the search for life on Mars.

We propose that future lander and rover missions to Mars should be equipped with cleaving and optical imaging capabilities with magnification up to 2000×. These tools would allow for martian gypsum and other salt minerals to be optically examined in situ for microfossils. This optical examination might provide preliminary evidence of life and would be useful in targeting specific samples for more advanced methods, such as laser Raman spectroscopy, which would lead to chemical and biological identification of organic materials.

Recent studies have suggested that Mars has, at times, been habitable (i.e., Squyres et al., 2012). However, the recognition of some microenvironments, such as fluid inclusions in halite and gypsum, has been overlooked. Our sedimentological and petrographic observations of gypsum from a Mars-analog setting demonstrate the remarkable ability of these minerals to trap and preserve microbiological communities. This leaves open the possibility that there may be a living microbiological community on Mars today.

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